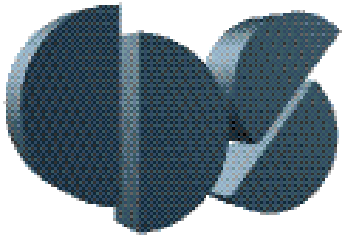
TX-TL Workshop
26-30 Aug 2013



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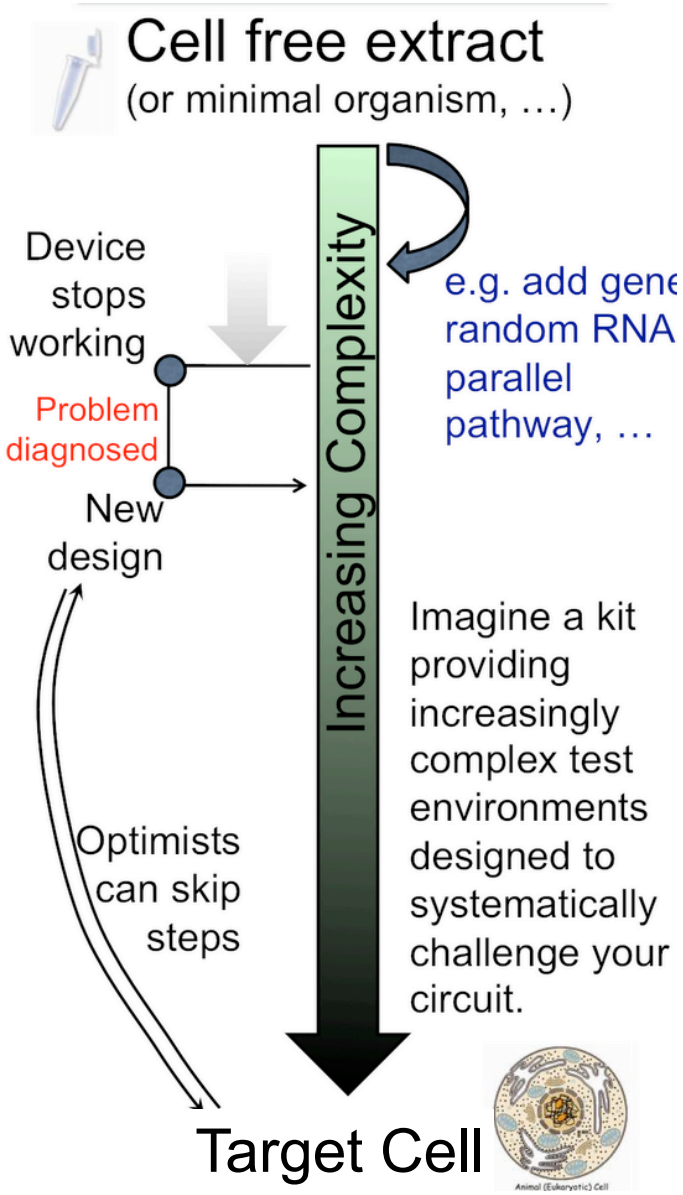
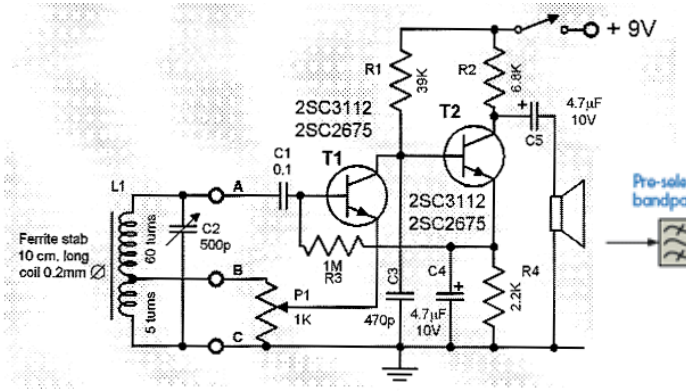
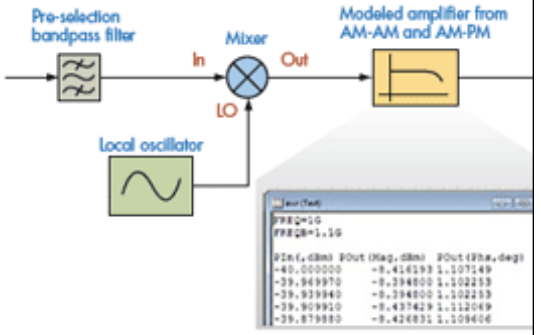
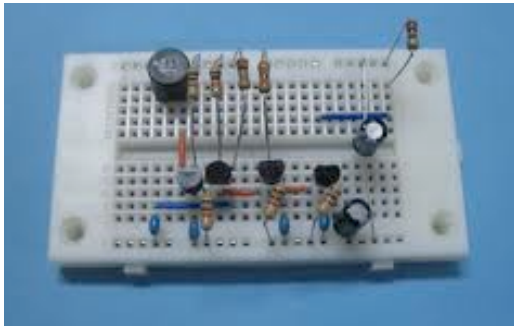
Sponsored by: DARPA Living Foundries (HR0011-12-C-0065)

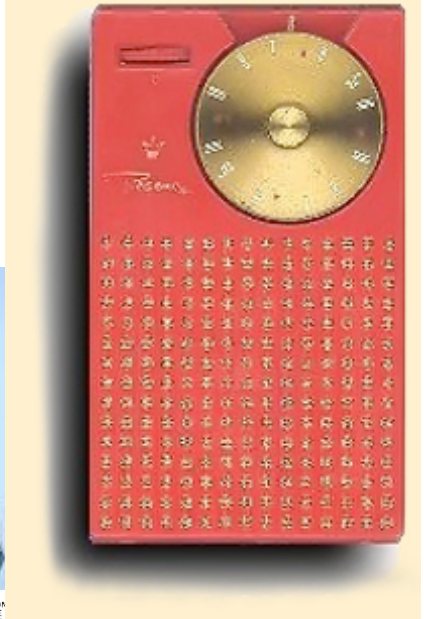
Biomolecular Breadboarding ("Wind Tunnel" [Klavins])







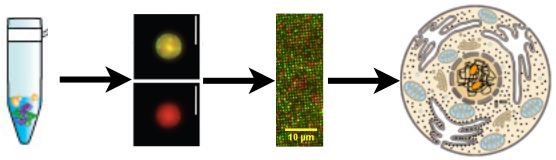




Biomolecular Breadboards

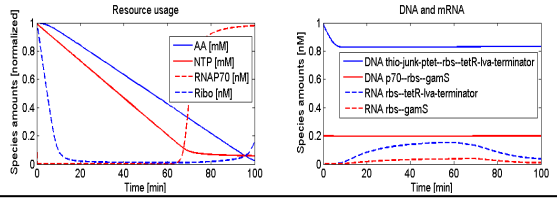
TX-TL cell-free toolbox

- $0.03/ul, 1 day cycle time
- Linear DNA (w/ protect'n)
- Protein degradation (via YbaQ and ssrA tags)
- Detailed protocols (JoVE)
- Circuits: switch, IFFL, toxin-antitoxin, RNA logic
- CSHL course in Jul 2013



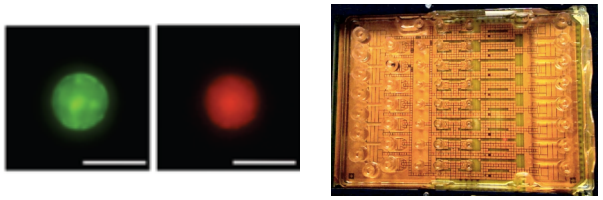
TX-TL modeling toolbox

- MATLAB (Simbiology) based toolbox with 10 line circuit specs
- Validated models for gene expression, regulation, w/ resource lims
- Full source code and user documentation available on web



TX-TL vesicles & droplets

- Inducer-based expression in vesicles, droplets
- Time course measurements of circuits in 0.3 µl droplets on Liquid Logic microfluidic platform
- Recent: protocols for mixing, merging, splitting plus improved imaging



Open source information

- TX-TL protocols, data, tools: http://www.openwetware.org/wiki/breadboards Sun et al, JoVE 2013
- TX-TL modeling library: http://www.sourceforge.net/projects/txtl Tuza et al, CDC 2013 (s)
- TX-TL announcements mailing list: http://groups.google.com/d/forum/txtl-announce

Sample TX-TL Based Design Process

S0: modeling (minutes/cycle, systematic design & analysis)

- Desired function + specs → set of possible designs (circuits) + sensitivity analysis
- Goal at this stage is to determine what circuits to test in TX-TL and predict outputs

S1: linear DNA (4-8 cycles @ 2 cycles/day, 24-96 variants)

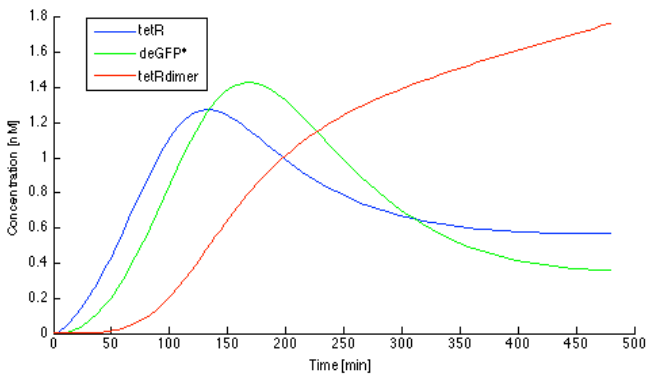
- Components from std library or PCR extension (no cloning)
- Test in TX-TL with GamS, ClpX. Try multiple circuits + vary ratios of copy numbers (based on achievable copy #'s)
- Compare w/ models; insure we can model what we see
- Goal: downselect 4-8 designs to test in plasmids

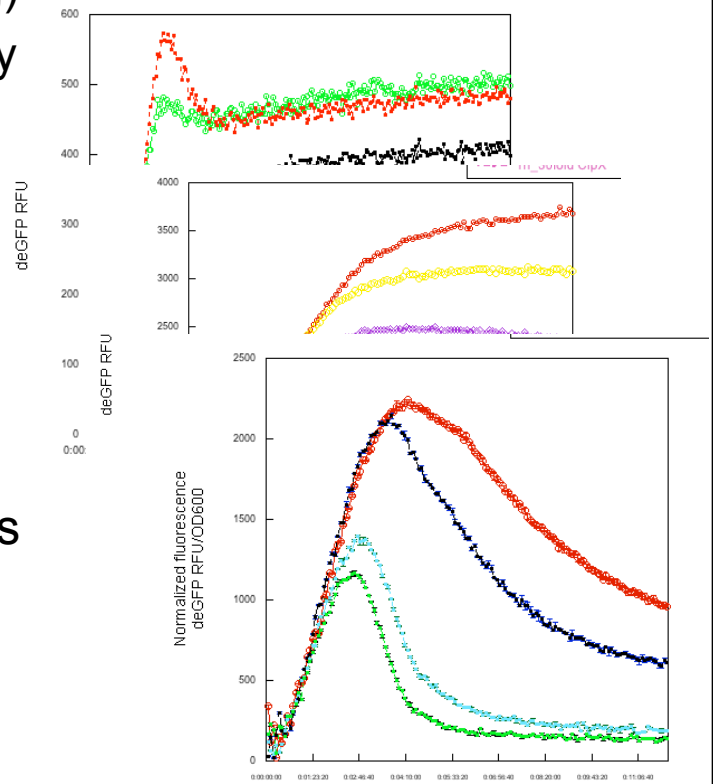
S2: plasmids (2-4 cycles @ 2 days/cycle, 8-24 variants)

- Clone into plasmid(s), using std sequences/protocols
- Verify operation in TX-TL, incl copy number variability
- Test robustness in multiple extracts w/ varying conditions
- Match results to S0 models and S1 linear DNA

S3: validate in cells (1 cycle, 4 days, 1-4 variants)

- Test top constructs from plasmid-based TX-TL assay





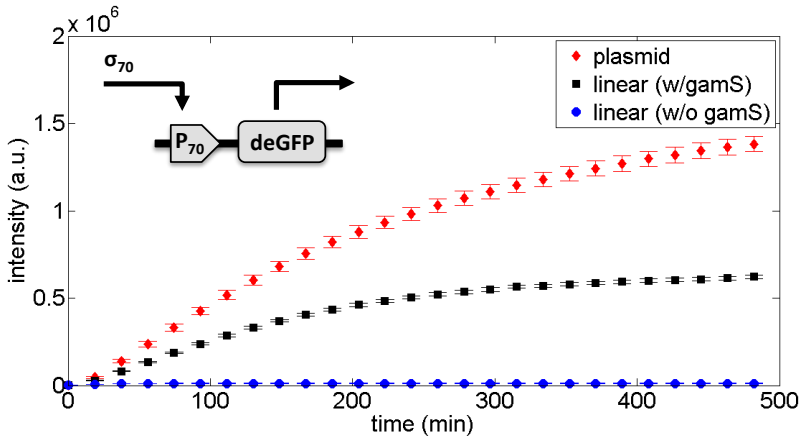
Sun, Hayes et al, 2013 (in preparation)

TX-TL Core Processes

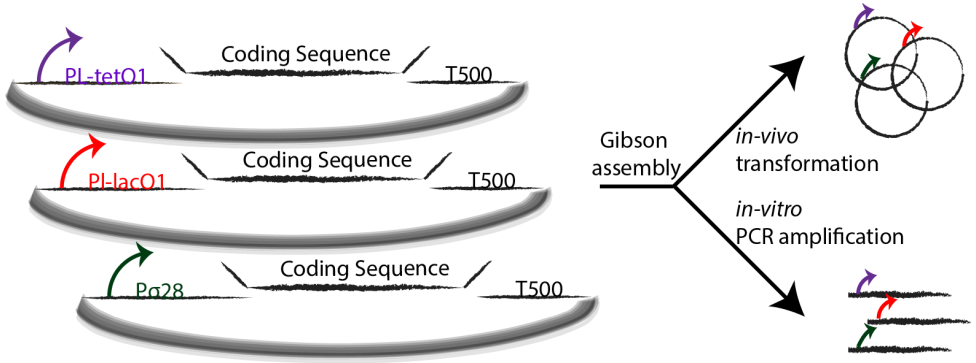
Zachary Sun, Vincent Noireaux

Rapid prototyping using linear DNA

- Use PCR products with GamS to get expression levels of ~60% of plasmid

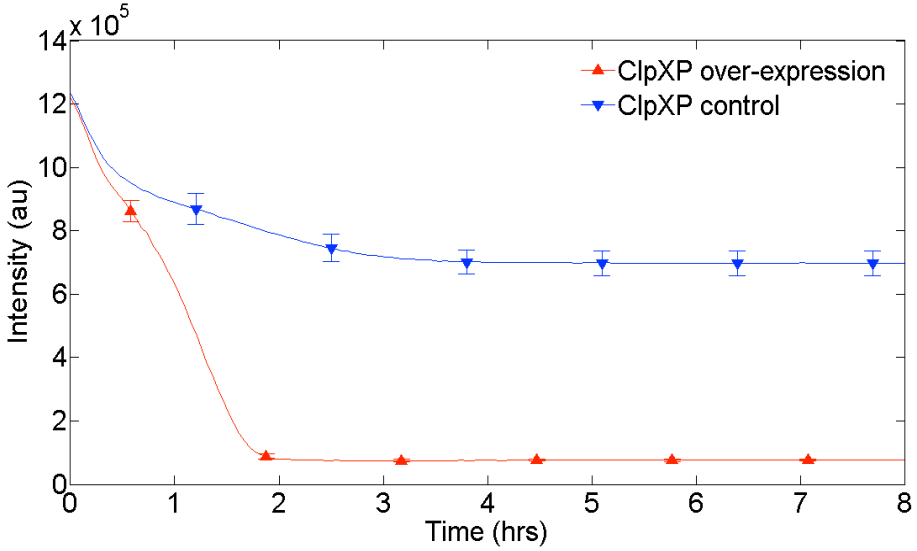


- Allows rapid assembly of constructs
  - PCR extension for simple circuits
  - IDT gBlocks + isothermal ass'y



Protein degradation

- Use clpXP machinery to degrade tagged proteins

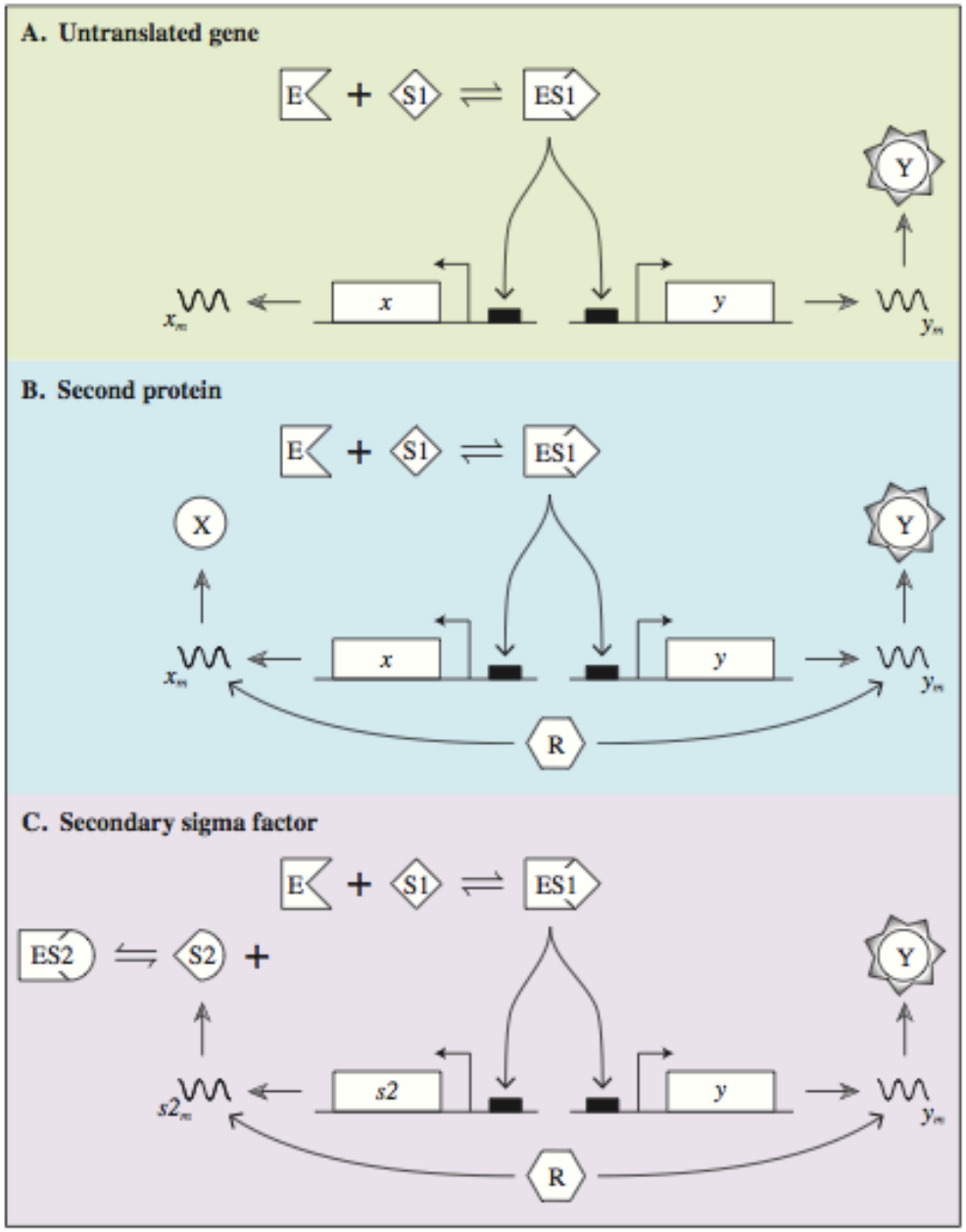


Tested components

- RNA polymerases: E. coli*, T7
- Activators: sigma28*, AraC*
- Repressors: TetR*, LacI*
- Reporters: deGFP*, MG, mSpinach
- Phosphorylation: NRI/pglnA
- DNA/RNA/protein deg: gamS*, clpXP*

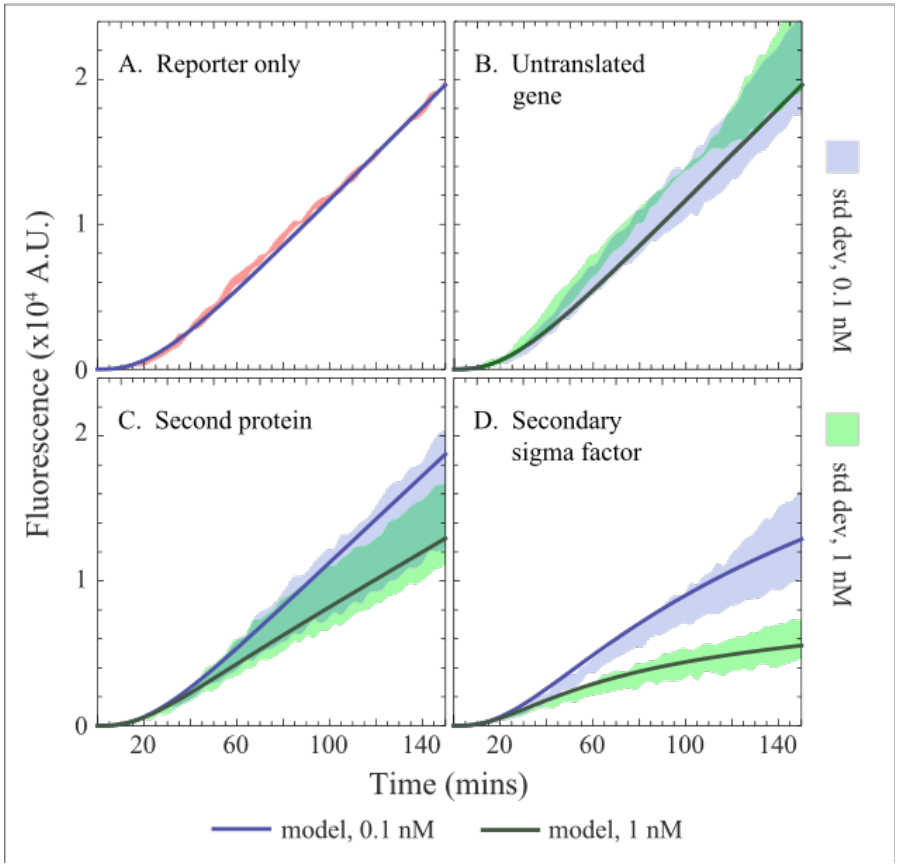
* preliminary models also available

Effects of Resource Limits



Which resources are limited?

- No evident transcriptional limits [B]
- Limited protein resources (AA, ATP) generate significant coupling [C]
- Sigma factors sequester RNAP [D]

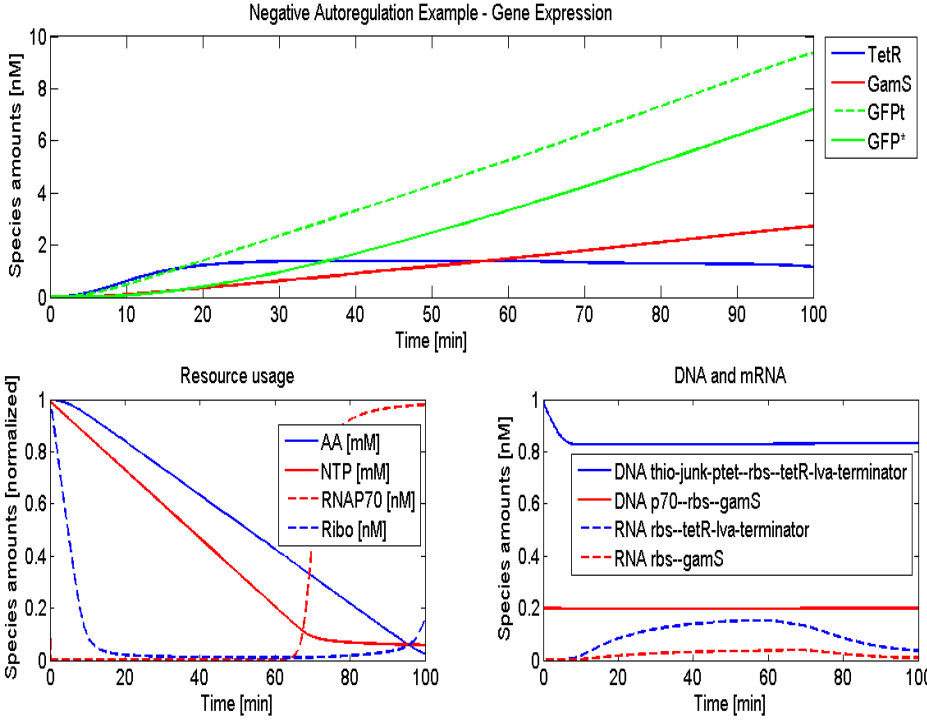


TX-TL Modeling

Zoltan Tuza, Vipul Singhal, Dan Siegal-Gaskins

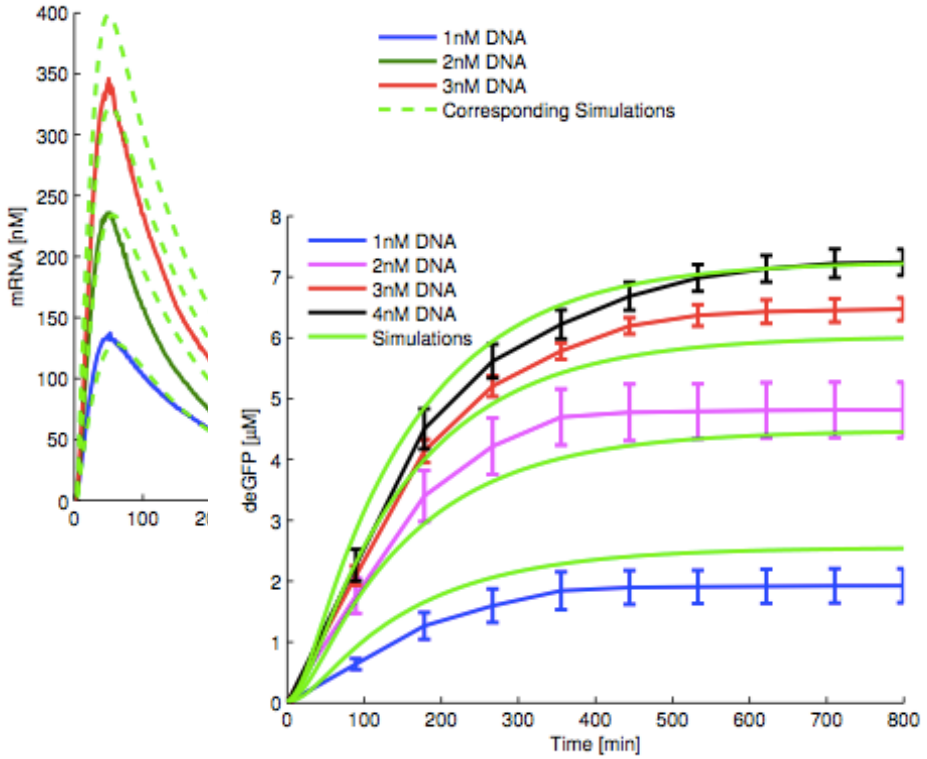
MATLAB toolbox (sf.net/projects/TXTL)

```
% Set up the standard TXTL tubes
tube1 = txtl_extract('e1');
tube2 = txtl_buffer('b1');

% Set up a tube that will contain our DNA
tube3 = txtl_newtube('circuit');
dna_tetR = txtl_dna(tube3, 'ptet', 'rbs', 'tetR', 100, 'linear');
dna_gamS = txtl_dna(tube3, 'p70', 'rbs', 'gamS', 10, 'plasmid');

% Mix the contents of the individual tubes and add some inducer
well_a1 = txtl_combine([tube1, tube2, tube3], [6, 2, 2]);
txtl_addspecies(well_a1, 'aTc', 0.1);

% Run a simulation
[t_ode, x_ode, names] = sbiosimulate(well_a1);
```



Resource utilization effects

- Model+TXTL shows effects of fixed number of RNAPs and ribosomes
- Additional sigma factor gene introduces significant 'crosstalk', reduces output
- Calibrated models that match experimental results



External TX-TL Circuit Testing

Circuit testing (DARPA LF, ONR MURI)

- Stage 1: you send us cells/plasmids; we verify *in vivo* operation (in our hands)
- Stage 2: we perform TX-TL runs, compare to *in vivo*, send you back data
- Stage 3: extended TX-TL modeling and characterization (joint activity)

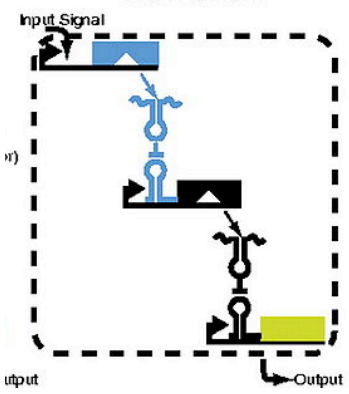
Things that work

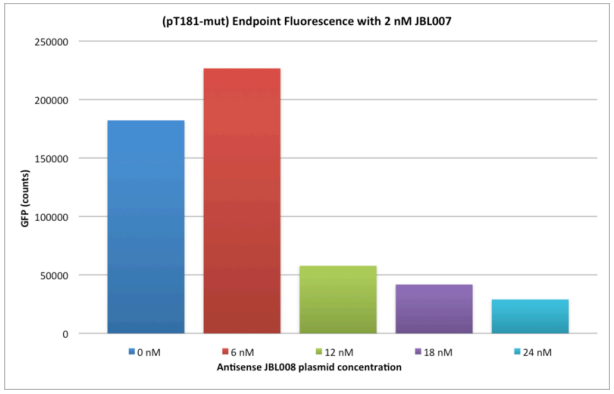
- Transcriptional circuits: neg autoreg, genetic switch, feedforward loops, logic
- RNA-based circuits (sometimes)
- Phosphorylation circuits (NRI)
- Metabolic pathways (2,3 BDO)

Things that haven't worked (yet)

- Green light sensor (??)
- Multi-layer cascades (resource lims)
- DNA integrase/excisionase (copy #?)
- Modified T7 RNAP (leaky expression)

PI (+ contact)	Circuit/Technology	1 2 3
Lucks (CH)	RNA-sensing TFs	✓✓✓
Del Vecchio (EY)	Loading effects	✓✓✓
Temme (VH)	Orthogonal RNAPs	✓? -
Voigt (DSG)	4 input, 11 gene	✓x -
Tabor (JK)	Green light sensor	✓✓○
Endy (VH)	DNA memory	✓○ -
Del Vecchio (SG)	Phospho-insulator	✓✓✓
Kortemme (EdlS)	Molecular sensors	✓○ -
Jewett (YW)	Butanediol pathway	✓✓○





TX-TL Limitations: Lessons Learned/Future Research

Resource limitations must be taken into account

- Easy to overload TX-TL machinery and create crosstalk
- Extend *duration* via "feeding solution", but still limited
- Models capture limits => should be able to avoid

Linear ≠ plasmid, *in vitro* ≠ *in vivo*

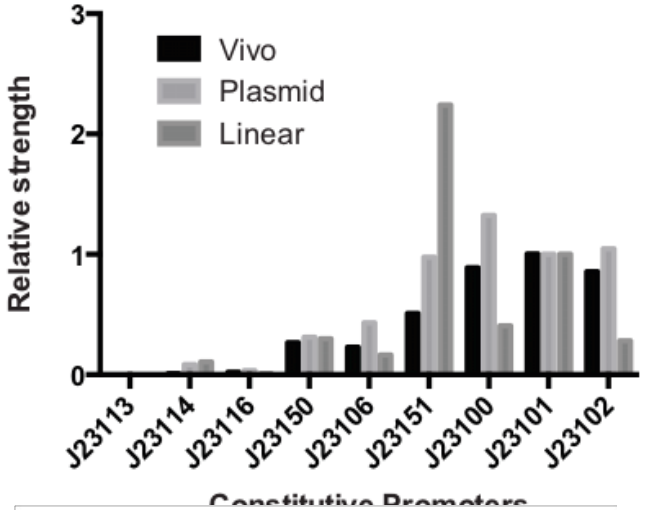
- Gene expression is dependent on DNA context
- OK for simple expression, but TX circuits require care
  - Just scaling up DNA concentration won't be enough
  - Use models to map between environments?
- Also: temperature, salts, co-factors and other effects
  - Eg: RNA structure depends on temp, [MG]/[K], ...

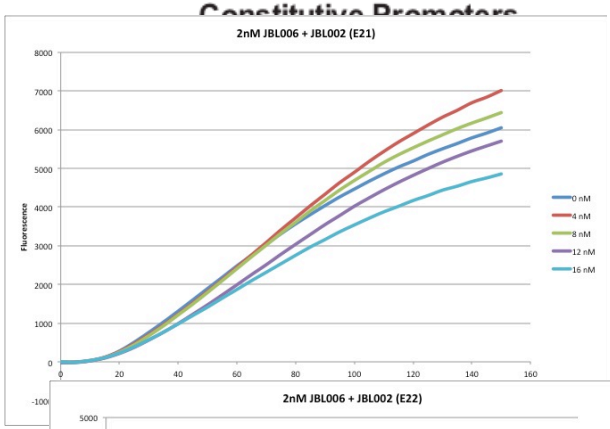
Batch-to-batch variations can create problems

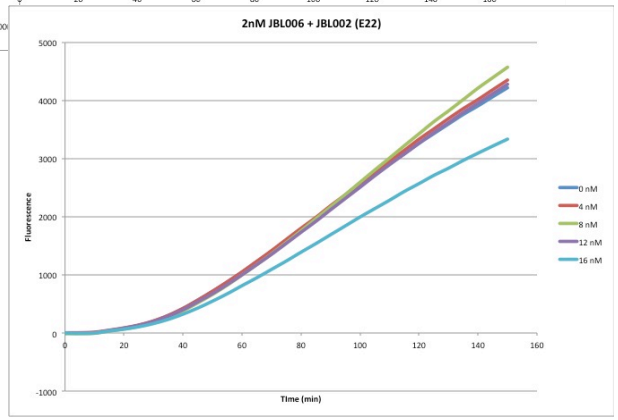
- Typically see 2X differences in expression levels between batches; sometimes different dynamics
- Some circuits that work in one batch don't work in others

Some circuits not yet working at all

- Green-light sensor (Tabor) - co-factors?



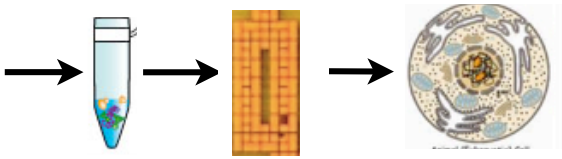




Biomolecular Breadboard Suite

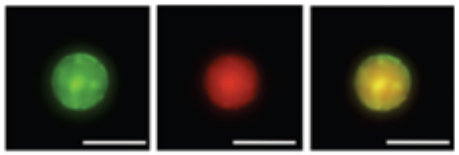
Cell-free breadboard

- Linear DNA assembly (build on work of others)
- Implemented ~8 circuits
- Document'd design cycle times (vs std cloning)
- Extract preparation video (Sun et al, JoVE, 2013)
- Predictive models for switch, IFFL, neg fbk



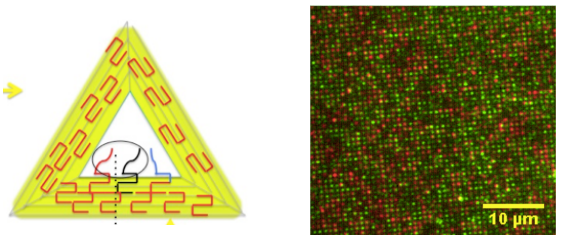
Artificial Cells

- Kinetics of expression inside vesicles
- Statistics of expression and induction (% of vesicles induced)
- Expression (and induction) as a function of vesicle size (1-100 fL)



Spatial Localization

- Control spatial location of DNA, RNA, proteins using DNA origami
- Explore effects of distance on hybridization, binding, scaffolding
- Demo'd transcription of bound DNA



Prototyping and debugging of *in vivo* and *in vitro* circuits

- Very little knowledge/infrastructure required to build *in vitro* circuits (try it!)
- Exploring use for synthetic biology courses (1 week labs); prototype at CSHL '13

Open source information

- TX-TL protocols, data, tools: http://www.openwetware.org/wiki/breadboards